http://dx.doi.org/10.5012/bkcs.2013.34.4.1272

Cytotoxicity of Substituted Benzimidazolyl Curcumin Mimics Against Multi-Drug Resistance Cancer Cell

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Received December 26, 2012, Accepted January 21, 2013

Key Words : Curcumin, Benzimidazole, Cytotoxicity, Multi-drug resistance, Curcumin mimics

Curcumin (diferuloyl methane, **1**), a major constituent of the root of *Curcuma longa* L., has useful biological properties, which are antiinflammatory,[1] antioxidant,[2] antiviral,[3] chemopreventive,[4] *anti*-infective activity,[5] and wound-healing properties.[6] We have previously reported that various alkyl amide and aryl amide linked curcumin mimics (**2**) have an angiogenesis inhibition effect[7] and curcumin derivatives (**3**) possessing diverse sulfonyl amide-linked functionalities exhibit a vasodilatation effect on the basilar artery of white rabbit induced by high K^+ ion.[8] In addition, we discovered that substituted triazolyl curcumin derivatives (**4**) synthesized from Cu(I)-catalyzed Huisgen 1,3-cycloaddition exhibit moderate to strong inhibitory activity against the osteoclastogenesis induced by the receptor activator of NF-κB ligand (RANKL),[9] which means that curcumin mimics with triazole groups will be potential drug candidates for treating osteoporosis. Based on the screening results for all curcumin mimics in our in-house library, their diverse biological properties mainly depend on the additional functional groups attached to the feruloyl structure and they generally exhibit mild cytotoxicity. However, very recently, we discovered that introduction of benzimidazole groups (**5**) to the feruloyl scaffold through aldol condensation greatly improved the cytotoxicity of newly synthetic curcumin mimics library (**6**) against various cancer cells.[10] Because the benzimidazole molecules exhibit a variety of biological properties such as anticancer,[11] antiviral,[12] and *anti*-hypertensive activity,[13] we can expect their novel cytotoxic properties come from benzimidazolyl groups attached to the feruloyl structure.

To date, the most efficient tool to treat cancer, viral infection, and so on is chemotherapy, but the occurrence of drug resistance due to clinically used small molecular drugs has become a significant obstacle in cancer and antiviral treatment.[14] In particular, because multidrug resistance (MDR) of cancer cells caused by prolonged administration of a certain drug can result in resistance toward multiple drugs, the cure rate and survivability of cancer patients by chemotherapy is critically reduced. Therefore, it is very urgent to study the recurrence of MDR and discover novel anticancer agents inhibiting MDR cells. Recently, curcumin and its related natural products were reported to modulate the human MDR1 gene expression.[15-17] We also previously reported that amide-linked curcumin derivatives (**2**) showed a potent MDR reversal activity by inhibiting drug efflux function of P-glycoprotein (P-gp).[18,19] When we are considering the our previous studies on the cytotoxicity against cancer cells and inhibitory effect on drug efflux function of P-gp of curcumin mimics library, we can anticipate the recent curcumin library

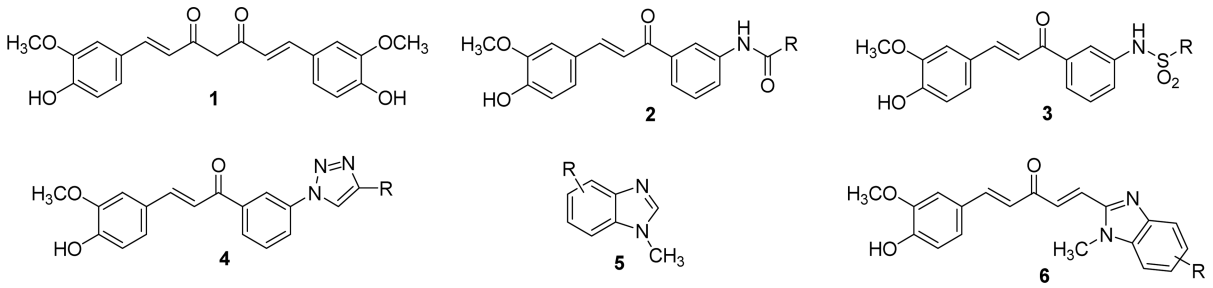
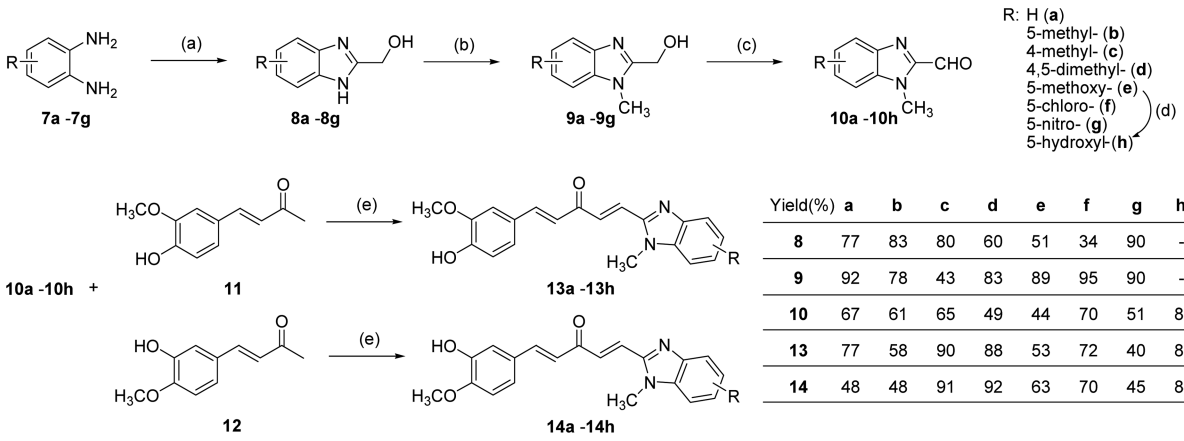


Figure 1. Structures of curcumin and synthetic curcumin mimic derivatives.

[a]These authors contributed equally to this work.



Yield(%)	a	b	c	d	e	f	g	h
8	77	83	80	60	51	34	90	-
9	92	78	43	83	89	95	90	-
10	67	61	65	49	44	70	51	82
13	77	58	90	88	53	72	40	85
14	48	48	91	92	63	70	45	84

Scheme 1. *Reagents and conditions*: (a) Glycolic acid (3 eq), 4 N HCl, reflux, 6 h; (b) Iodomethane (3 eq), tetrabutylammonium bromide (0.2 eq), potassium hydroxide (1.1 eq), tetrahydrofuran, 0 °C; (c) Dess-Martin periodinane (1.1 eq), methylene chloride, 0 °C, 6 h; (d) Tribromoborane (2 eq), methylene chloride, −78 °C; (e) **11** or **12** (each 1 eq), 40% KOH, Ethanol, ice bath, 10 h.

(**6**) possessing benzimidazole functionalities may inhibit the MDR cancer cell. In order to prove our postulation, we compared the cytotoxicity of curcumin derivatives (**6**) against multidrug resistant ovarian cancer cell lines with over-expressed P-gp and its origin ovcar-8 without P-gp and will report the result in this paper.

We synthesized benzimidazolyl curcumin mimic library (**5**) shown in Scheme 1. The reaction of variously substituted 1,2-phenylenediamine (**7a-7g**) with glycolic acid (3 eq) in 4 N hydrochloric acid produced substituted benzimidazolyl methanol (**8a-8g**) after reflux for 6 h.[20] The benzimidazolyl methanols **8a-8g** were reacted with iodomethane (3 eq) in the presence of tetrabutylammonium bromide (TBAB, 0.2 eq) and potassium hydroxide (KOH, 1.1 eq) in tetrahydrofuran (THF) at 0 °C to afford 1-methylbenzimidazolyl compounds (**9a-9g**),[21] which were subsequently oxidized with the Dess-Martin periodinane in methylene chloride at 0 °C to produce substituted benzimidazolyl 2-carbaldehyde (**10a-10g**).[22] 5-Hydroxy benzimidazolyl 2-carbaldehyde (**10h**) was also obtained from the demethylation of **10e** using tribromoborane (BBr_3, 2 eq) in methylene chloride at −78 °C.[23] The curcumin mimic library like **13** and its constitutional isomeric curcumin derivatives such as **14** were obtained from the aldol reaction of **11** and **12** with synthetic benzimidazolyl 2-carbaldehyde compounds (**10a-10g**), respectively.[8] The instrumental data of curcumin library confirmed through ^{1}H NMR and ^{13}C NMR spectroscopy were already reported in our previous report.[10]

To evaluate the cytotoxicity of novel curcumin mimics with structurally diverse benzimidazol moieties against MDR cancer cells, we conducted a cytotoxicity assay by employing the MTT colorimetric method[24] against NCI/ADR-RES cell with over-expressed P-gp and OVCAR-8 without P-gp.[25] The cytotoxicity of benzimidazolyl curcumin mimic library (**13a-13h** and **14a-14h**) against non-MDR cancer cells OVCAR-8 shows quite strong to moderate potency, namely is much stronger than their mother compound, curcumin. It means that the addition of the benzimidazole group to feruloyl structure dramatically increases the inhibitory potency on cancer cells by about 5-340 times with the IC_{50} of curcumin (**1**), indicating that we can design novel drug candidates from the adequate addition of diverse functional

Table 1. Inhibitory concentration of curcumin mimic library against various cancer cell lines

Entry		Cancer cells (IC_{50} μM)[a]		RF[b]	Entry		Cancer cells (IC_{50} μM)[a]		RF[b]
		NIH/ADR-RES	OVCAR-8				NIH/ADR-RES	OVCAR-8	
13	**a**	21.3	5.1	4.2	**14**	**a**	23.7	5.4	4.4
	b	9.6	5.6	1.7		**b**	9.8	5.7	1.7
	c	23.2	0.7	33.1		**c**	443.7	72.2	6.1
	d	79.5	50.7	1.6		**d**	45.2	6.8	6.6
	e	35.0	6.5	5.4		**e**	30.8	5.9	5.2
	f	24.0	6.3	3.8		**f**	28.5	6.4	4.5
	g	29.2	6.2	4.7		**g**	117.6	59.9	2.0
	h	18.4	5.8	3.2		**h**	8.2	5.7	1.4
curcumin		854.5	238.5	3.6	Paclitaxel		70.0	0.005	14000
vincristine		138.5	0.001	138500					

[a]IC_{50} was calculated from nonlinear regression by Graphpad Prism software. [b]Resistance factor (RF) to each drug was calculated as the ratio of the IC_{50} values of NIH/ADR cells to that of OVCAR-8 cells

groups to feruloyl structure. In order to clarify the *anti*-MDR cancer cell activity of our curcumin library, we test the same screening using MDR ovarian cancer cell (NCI/ADR-RES). As shown in Table 1, the inhibitory effect on MDR ovarian cancer cell is weaker than in case of test on OVCAR-8. However, when considering the resistance factor (RF)[26] which calculate as the ratio of the IC_{50} values of NIH/ADR cells to that of OVCAR-8 cells, benzimidazolyl curcumin mimic library (**13a-13h** and **14a-14h**) shows a small RF value, which means that the difference of the inhibitory potency between MDR ovarian cancer cell (NCI/ADR-RES) and non-MDR ovarian cancer cell (OVCAR-8) is narrow and they may be good candidates for treating cancer cells with MDR. Most important anticancer drugs such as paclitaxel and vincristine exhibit 140,000 and 138,500 values, respectively. In particular, **13b** and **14b** have a strong cytotoxic effect on both each ovarian cancer cells and low RF value (each 1.7) and **14h** also shows a strong inhibitory effect and 1.4 of RF value. In case of **13c**, its inhibition effect is quite strong (IC_{50} = 23.2 μM on NCI/ADR-RES but 0.7 μM on OVCAR-8) but relatively high RF value (33.1). Thus it could not differentiate MDR cancer cell from non-MDR cell. Based on the screening results of Table 1, because curcumin have a weak anticancer activity but low RF value, it is possible to discover novel anticancer drug candidates by structurally modifying curcumin mimics that have an inhibitory properties against cancer cells with multi-drug resistance. When considering that our previous amide-linked curcumin mimics (**2**) inhibit drug efflux function of P-gp and increase accumulation of anticancer drugs in the cancer cells,[18,19] it is possible to postulate that feruloyl group contribute to decrease the drug efflux function of P-gp and benzimidazole group increase the potency of cytotoxicity.

In conclusion, a novel curcumin mimics library (**13a-13h** and **14a-14h**) through the aldol reaction of (*E*)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (**11**) or (*E*)-4-(3-hydroxy-4-methoxyphenyl)but-3-en-2-one (**12**) with diversely substituted benzimidazolyl-2-cabaldehyde (**10a-10h**) have been synthesized with the purpose of discovering novel anticancer drug candidates that inhibit a proliferation both MDR cancer cells and non-MDR cancer cells at the same time. On the basis of the MTT assay against the cancer cells on NCI/ADR-RES and OVCAR-8, we confirmed that our novel curcumin mimics inhibit the growth of MDR cancer cells more strongly than our previous in-house curcumin mimic libraries and curcumin. Among the tested derivatives, **13b**, **14b**, and **14h** are the strongest candidates inhibiting multi-drug resistance cancer cells.

Acknowledgments. This work was supported by a research grant from Yonsei University Wonju College of Medicine (YUWCM 2012-58). The authors also thank for the technical support from Natural Medicine Center at Korea Institute of Science and Technology.

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